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Yamamoto et al., Nature 319: 230-234 1986

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Thanks,

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## An Alternatively Processed mRNA from the Avian *c-erbB* Gene Encodes a Soluble, Truncated Form of the Receptor That Can Block Ligand-Dependent Transformation

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At least four major transcripts are produced by the avian *c-erbB*/epidermal growth factor receptor gene. cDNAs corresponding to the smallest one, a 2.6-kb transcript, were isolated from an adult chicken liver cDNA library. Sequence analysis revealed that the 3' end of one cDNA clone diverged from the known sequence of the extracellular ligand-binding domain (LBD) of the full-length receptor. A genomic DNA subfragment that contained this unique 3' divergent end was isolated. Sequence analysis of this genomic DNA fragment revealed that the 2.6-kb *c-erbB* transcript is produced by alternative processing. Translation of this 2.6-kb transcript would produce a secreted, truncated receptor molecule which contains the amino-terminal three-fourths of the extracellular LBD of the native receptor. COS1 cells and primary chicken embryo fibroblast cells were transfected with expression vectors that contained the 2.6-kb *c-erbB* cDNA. Conditioned medium from these transfected cells contained a 70-kDa protein that was specifically immunoprecipitated by a polyclonal antiserum directed against the LBD of the avian *c-erbB* gene product. The 70-kDa truncated receptor could be coimmunoprecipitated from conditioned medium of transfected COS1 cells that was supplemented with recombinant human transforming growth factor alpha (TGF $\alpha$ ) by a monoclonal antibody against human TGF $\alpha$ . Additionally, transfected chicken embryo fibroblast cells that overexpressed the 70-kDa truncated receptor were blocked in their ability to form TGF $\alpha$ -dependent colonies in soft agar. These data suggest that the secreted, truncated receptor encoded by the 2.6-kb *c-erbB* transcript can bind to TGF $\alpha$  and may play an important growth-regulatory function in vitro.

Growth factors are an important group of regulatory polypeptides that govern cell proliferation and differentiation through their cognate cell surface receptors. Several growth factor receptors possess an intrinsic cytoplasmic tyrosine kinase activity that is stimulated to undergo autophosphorylation and phosphorylation of other protein substrates by growth factor binding to the extracellular domain of the receptor (reviewed in references 13, 64, and 58). One member of this family of growth factor receptor tyrosine kinases is the human epidermal growth factor (EGF) receptor (EGF-R). The human EGF-R is a 170-kDa transmembrane glycoprotein that can bind to at least four different ligands: EGF, transforming growth factor alpha (TGF $\alpha$ ), vaccinia virus growth factor, and to a lesser extent amphiregulin (11, 14, 52, 56). The human EGF-R has been cloned, and sequence analysis has demonstrated this receptor to be the cellular homolog of the avian *v-erbB* oncogene (19, 57, 63). The avian *c-erbB* proto-oncogene has recently been isolated and shown to encode a 170-kDa transmembrane glycoprotein that is structurally homologous to the human EGF-R (32). Interestingly, the chicken EGF-R homolog encoded by the avian *c-erbB* gene appears to be functionally distinct from the human EGF-R since it preferentially binds TGF $\alpha$  with an approximately 200-fold-higher affinity than it binds EGF (32). The chicken EGF-R homolog has also been found to act as a hematopoietic growth factor receptor that regulates self-renewal of normal chicken erythroid progenitor cells during hematopoiesis, while human EGF-R gene products

appear to be absent from hematopoietic cells (43). Another distinguishing feature is that the normal avian *c-erbB* gene, along with the normal rat EGF-R gene (44), produce a small RNA transcript that encodes a secreted, truncated receptor, whereas an analogous form of the human EGF-R gene has not yet been reported. A similar transcript, however, has been reported in the human epidermoid carcinoma cell line A431, in which the EGF-R gene is amplified and rearranged (57, 63). Synthesis of the 2.8-kb transcript in A431 cells is due to a genomic rearrangement resulting in the fusion of the extracellular ligand-binding domain (LBD) of the EGF-R to an unidentified region of genomic DNA (40).

Soluble, truncated receptors (i.e., receptors lacking their transmembrane and cytoplasmic domains) are not unique to the EGF-R family. In fact, soluble, truncated receptors have recently been reported for ligands as diverse as acidic and basic fibroblast growth factor (FGF) (27), colony-stimulating factor (CSF)-1 (18), granulocyte CSF (22), granulocyte-macrophage CSF (3, 47), growth hormone (GH) (8, 33), insulinlike growth factor II (36), interleukin-2 (IL-2), IL-4, IL-5, IL-6, and IL-7 (23, 35, 41, 49, 54), lutropin/choriogonadotropin (34), nerve growth factor (17), transferrin (9), and tumor necrosis factor (51). Two alternate mechanisms appear to be responsible for the generation of these soluble, truncated receptors. One mechanism of generating truncated receptor molecules appears to involve proteolytic processing of full-length receptors. In the case of the CSF-1 receptor, formation of a soluble, truncated receptor is by proteolytic cleavage and is a down-modulatory response to ligand binding and protein kinase C activation (18). In contrast, the other mechanism involves alternative splicing or processing

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of the primary RNA transcripts from these receptor genes. This group of soluble, truncated receptors includes the chicken EGF-R homolog, the rat EGF-R (44), the FGF receptor (27, 28), the granulocyte CSF receptor (22), the granulocyte-macrophage CSF receptor (3, 47), the GH receptor (8, 33), the IL-4 receptor (41), the IL-5 receptor (54), the IL-6 receptor (35), the IL-7 receptor (23), and the lutropin/choriogonadotropin receptor (34).

Here, we report the cloning of a small, 2.6-kb transcript from the avian *c-erbB* gene from an adult chicken liver cDNA library that encodes a truncated, soluble form of the native receptor. We also provide evidence that this 2.6-kb transcript is produced by alternative processing. The truncated *c-erbB* transcript and the transcripts encoding the full-length receptor appear to be coexpressed in most chicken embryonic tissues and in a variety of normal adult tissues. The 2.6-kb transcript produces a secreted, 70-kDa protein when transfected into COS1 and chicken embryo fibroblast (CEF) cells. The 70-kDa protein encoded by the 2.6-kb transcript is structurally distinct from the proteins encoded by the 2.8-kb transcript in human A431 cells and the 2.7-kb transcript in normal rat tissue. We also demonstrate that the 70-kDa truncated receptor secreted from transiently transfected COS1 cells can bind to TGF $\alpha$ , as demonstrated by coimmunoprecipitation with a nonneutralizing monoclonal antibody against TGF $\alpha$  (MAB-TGF $\alpha$ ). Furthermore, we demonstrate that stably transfected CEF cells which overexpress the 70-kDa truncated receptor are blocked in their ability to form TGF $\alpha$ -dependent colonies in soft agar. The identification of soluble truncated receptor forms in many receptor systems suggests that these proteins may share some physiological function.

## MATERIALS AND METHODS

**Cell culture.** CEF (line 0) cells were obtained from the Avian Disease and Oncology Laboratory, U.S. Department of Agriculture, East Lansing, Mich. The CEF cells were maintained in Dulbecco modified Eagle medium (DMEM) containing 4.5 g of glucose per liter and supplemented with 4% fetal bovine serum (FBS), 1% chicken serum, 50 U of penicillin per ml, and 50  $\mu$ g of streptomycin per ml (all from GIBCO). COS1 cells (American Type Culture Collection) were maintained in DMEM supplemented with 8% FBS, 50 U of penicillin per ml, and 50  $\mu$ g of streptomycin per ml. All cells were cultured in 5% CO<sub>2</sub>-humidified air at 37°C.

**Isolation of cDNA clones.** Duplicate sets of nitrocellulose (Millipore) plaque lifts were prepared from approximately 10<sup>6</sup> plaques of an adult chicken liver cDNA library in  $\lambda$ ZAP (Stratagene). These duplicate filters were separately hybridized by standard procedures (4) to <sup>32</sup>P-labeled cDNA fragments (38) from the extracellular LBD (probe 2; Fig. 1A) and the intracellular kinase domain (probe 1; Fig. 1A). Only two clones, L173 and L207.3, exclusively hybridized to probe 2. These clones were plaque purified, and the pBS(SK-) vectors with cDNA inserts were excised from  $\lambda$ ZAP as specified by the manufacturer (Stratagene).

**Identification of a genomic DNA clone.** Overlapping genomic  $\lambda$ EMBL clones of the avian *c-erbB* locus (45) were digested with *Eco*RI, separated on a 1% agarose-1 $\times$  Tris-borate-EDTA gel, and transferred (53) to Nytran (Schleicher & Schuell). The blot was hybridized with a <sup>32</sup>P-labeled fragment from the 3' divergent end of pBSL207.3 (probe 3; Fig. 1A). Probe 3 hybridized to a 2.7-kb *Eco*RI fragment which was gel purified and cloned into the *Eco*RI site of pBS(KS+) (Stratagene) to make pBSg2.7RI.

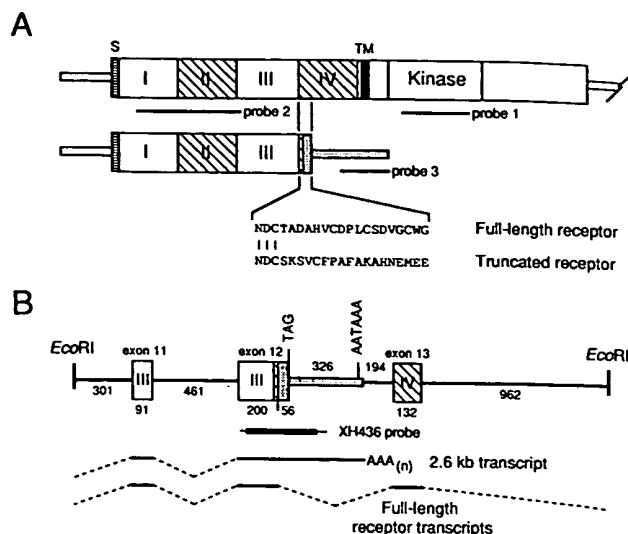


FIG. 1. Schematic diagram of the full-length and truncated receptors and a partial exon-intron map of *c-erbB*. (A) Comparison of the structures and amino acid sequences of the full-length and truncated receptors. I to IV, subdomains within the LBD; horizontally hatched box, signal peptide (S); diagonally hatched boxes, cysteine-rich domains; black box, transmembrane domain (TM); kinase, tyrosine kinase domain; stippled box, divergent region of the truncated receptor; smaller boxes, untranslated regions; probes 1 to 3, cDNA fragments used as probes (see Fig. 3). (B) Exon-intron map of a 2.7-kb *Eco*RI genomic fragment from *c-erbB*, with an illustration of the splicing pattern for the truncated and full-length receptor transcripts. The sizes of the exon-intron regions are shown in nucleotides. XH436 probe, a 358-nt *Xba*I-*Hinc*II fragment (thicker line) used to generate a 436-nt antisense RNA probe for RNase protection (see Fig. 4).

**DNA sequence analysis.** Nested deletions (26) of pBSL173, pBSL207.3, and pBSg2.7RI were prepared by using exonuclease III and mung bean nuclease (both from U.S. Biochemical). Both strands were sequenced by the dideoxy-chain termination method (50) with Sequenase, using dGTP reactions and dITP reactions as needed according to the protocol of the manufacturer (U.S. Biochemical).

**Isolation and analysis of RNA.** Total cellular RNA was extracted from cells by using either the guanidinium thiocyanate-cesium chloride method (15) or the guanidinium thiocyanate-phenol-chloroform extraction method (16). Poly(A)<sup>+</sup> RNA was selected twice by oligo(dT)-cellulose chromatography (5). Ten micrograms of CEF poly(A)<sup>+</sup> RNA per lane was electrophoresed in 1% agarose-3-(*N*-morpholino) propanesulfonic acid (MOPS)-formaldehyde gels and transferred (55) to Nytran (Schleicher & Schuell). The blots were hybridized separately to <sup>32</sup>P-labeled cDNA probes 1, 2, and 3 (Fig. 1A) and washed by standard procedures (4).

The RNase protection probe was made by subcloning a 358-nucleotide (nt) *Hinc*II-*Xba*I cDNA fragment that corresponded to a portion of exon 12 of the 2.6-kb transcript into the *Sma*I-*Xba*I sites of pBluescript (KS-; Stratagene). A 436-nt antisense RNA probe (XH436; Fig. 1B) was transcribed in vitro from an *Xho*I-linearized template, using [ $\alpha$ -<sup>32</sup>P]UTP (800 Ci/mmol; Amersham) and bacteriophage T7 RNA polymerase (U.S. Biochemical) according to standard procedures (4). Fifty micrograms of total RNA was denatured at 85°C for 5 min with 2.5  $\times$  10<sup>5</sup> cpm of radioactive antisense XH436 probe in 80% formamide-0.4 M sodium

chloride–40 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES; pH 6.5)–1 mM EDTA and hybridized overnight at 50°C. Unhybridized probe was removed by digestion with 6 U of RNase T<sub>2</sub> (Bethesda Research Laboratories) in 20 mM sodium acetate (pH 4.5)–300 mM sodium chloride for 45 min at 37°C. The RNase-resistant hybrids were first extracted with phenol-chloroform (50:50) and then ethanol precipitated and electrophoresed on a 6% polyacrylamide gel containing 7 M urea.

**Expression of the 2.6-kb cDNA in COS1 and CEF cells.** The 2.6-kb cDNA was first digested with *Sst*I, then repaired with T4 DNA polymerase, and digested with *Xba*I (all from U.S. Biochemical). The resulting 1,748-nt blunt-*Sst*I-*Xba*I cDNA fragment (2.6SX) was subcloned into a blunt-*Xho*I-*Xba*I-prepared pSVL expression vector (Pharmacia-LKB) or a blunt-*Hind*III-*Xba*I-prepared pRcCMV expression vector (Invitrogen). The transient eucaryotic expression vector pSVL is a pBR322-based plasmid designed for high levels of expression in COS cells. This vector contains the simian virus 40 (SV40) origin of replication, the SV40 late promoter, the SV40 VP1 intron followed by a multiple cloning site, and the SV40 polyadenylation signal. The stable eucaryotic expression vector pRcCMV is a pUC-based plasmid that utilizes a cytomegalovirus (CMV) promoter followed by a multiple cloning site and the bovine GH polyadenylation signal for high levels of expression in eucaryotic cells. Additionally, pRcCMV has the neomycin resistance gene under the control of the SV40 early promoter and the SV40 polyadenylation signal for selection of stably expressing cells. Twenty micrograms of pSVL2.6SX or pRcCMV2.6SX was transfected into ~60% confluent 100-cm<sup>2</sup> plates of COS1 or CEF cells, respectively, by the calcium phosphate-DNA precipitate method (61). COS1 cells and medium were analyzed 72 h after transfection for truncated receptor expression. The CEF cells were selected for 10 days in complete DMEM with G418 (0.8 mg/ml; ~50% active; GIBCO) and then maintained in complete DMEM with G418 at 0.4 mg/ml before analysis of truncated receptor expression or culture in soft agar.

**Immunoprecipitation and SDS-PAGE.** Approximately 5 × 10<sup>5</sup> COS1 or CEF cells were incubated for various periods of time (16 to 22 h) in RPMI 1640 medium lacking cysteine (GIBCO) supplemented with 2.5% dialyzed FBS (GIBCO) in the presence of [<sup>35</sup>S]cysteine (100 µCi/ml; 1,300 Ci/mmol; Amersham). For the analysis of N-linked glycosylation, 5 × 10<sup>5</sup> cells were preincubated in 1 ml of complete RPMI 1640 lacking cysteine and containing tunicamycin (1 µg/ml; Sigma) for 2 h before the addition of [<sup>35</sup>S]cysteine and processed in the same way as were the other cells. Samples of conditioned medium were removed and saved. Cells were washed once in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.4 mM KH<sub>2</sub>PO<sub>4</sub>) and then lysed in RIPA buffer (140 mM NaCl, 50 mM Tris [pH 8.0], 5 mM EDTA, 1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulfate [SDS]) plus protease inhibitors (5 µg of aprotinin per ml, 5 µg of soybean trypsin inhibitor per ml, 1 mM phenylmethylsulfonyl fluoride; all from Sigma) for 30 min at 4°C on a rocking platform. To the samples of saved conditioned medium (1 ml), 0.25 ml of 5× RIPA buffer (lacking sodium chloride) supplemented with protease inhibitors was added; samples were placed on a rocking platform at 4°C for 30 min. Insoluble material was pelleted by centrifugation at 16,000 × *g* for 10 min at 4°C, and the supernatant was removed to a clean 1.5-ml tube. Two microliters of the anti-LBD polyclonal serum (αLBD) (38) or preimmune control serum was added to the samples, which were rocked

overnight at 4°C. Then 25 µl of a 25% slurry of protein A-Sepharose (Pharmacia-LKB) was added, and samples were rocked at 4°C for 1 h. Protein A-Sepharose beads were washed three times with RIPA buffer plus protease inhibitor; and once with Tris-buffered saline (100 mM NaCl, 10 mM Tris [pH 7.6]). An equal volume of 2× sample buffer was added to samples, which were then boiled for 5 min and then subjected to polyacrylamide gel electrophoresis (PAGE) or a 0.1% SDS–7.5% polyacrylamide gel as described previously (29). The gels were treated with En<sup>3</sup>Hance (Du Pont NEN) before fluorography.

**Coimmunoprecipitation of the truncated receptor with TGFα.** Samples of <sup>35</sup>S-labeled conditioned medium, pre-cleared of insoluble material from 5 × 10<sup>5</sup> transfected or untransfected COS1 cells as described above, were used for these immunoprecipitations. The controls (preimmune serum and αLBD) were immunoprecipitated as described above except that protein G-Sepharose (Sigma) was substituted since it quantitatively binds the mouse monoclonal antibody used in the other samples. A sample of conditioned medium from pSVL2.6SX-transfected COS1 cells was first immunodepleted of avian *c-erbB* protein products by the addition of 2 µl of αLBD and 25 µl of a 25% slurry of protein A-Sepharose and rocked at room temperature for 1 h before the resulting supernatant was processed as described below. Coprecipitation samples (1 ml) were incubated with 1 ng of recombinant human TGFα (GIBCO) per ml for 30 min at room temperature, then 15 µl of MAb-TGFα (Ab-1; Oncogene Science) and 0.25 ml of 5× RIPA buffer (lacking sodium chloride) plus protease inhibitors were added, and the samples were rocked overnight at 4°C. Then 25 µl of a 25% slurry of protein G-Sepharose was added, and the samples were rocked at 4°C for 1 h. The samples were washed and analyzed by SDS-PAGE as described above.

**Soft agar colony formation assay.** CEF cells (10<sup>4</sup> cells per 35-mm well) stably transfected with pRcCMV2.6SX or pRcCMV were suspended in 2 ml of complete DMEM (0.5% chicken serum, 4.5% FBS) plus 0.35% Noble agar (Difco) and plated on a 2-ml cushion of complete DMEM plus 0.5% Noble agar. After 20 to 24 h, 2 ml of complete DMEM plus 0.35% Noble agar alone or supplemented with recombinant human TGFα (to give a final concentration of 0.05 to 50 ng/ml) was added to the suspended CEF cells. Two weeks later, the cells were photographed under a Nikon Diaphot microscope, using a 4× objective and Kodak technical pan film.

**Nucleotide sequence accession number.** The sequences of the cDNAs encoding the 2.6-kb *c-erbB* transcript (Fig. 2) and the 2.7-kb genomic *Eco*RI fragment (Fig. 1B) have been submitted to the GenBank data base under accession numbers M77637 and M77638, respectively.

## RESULTS

**Isolation and sequence of a *c-erbB* cDNA which encodes a truncated receptor.** We and others have previously shown that of the four major avian *c-erbB* transcripts detected with probes corresponding to the extracellular LBD, only the 2.6-kb transcript will not hybridize to probes from the intracellular tyrosine kinase domain (38, 42, 45; see below). To isolate cDNA clones encoding the avian *c-erbB* 2.6-kb transcript, approximately 10<sup>6</sup> plaques of an adult chicken liver cDNA library in λZAP (Stratagene) were screened with <sup>32</sup>P-labeled probes 1 and 2 (Fig. 1A). Only two cDNA clones, λL173 and λL207.3, hybridized exclusively to the extracellular LBD probe 2. Bluescript (Stratagene) plasmids

TAGTCCACG GCTGCCCGC GCTGCTCCG AAACCTGTG ACAGAAAGGA GCGCAGGAG GAGGAAAGAA GAGGAGGAGA AGGAAGGGGA	90
GGAGGAAGAA GAAGAGGAAG AAGGCAGCAG TCCGCCCGCA GCTGGGTAGG CACGTGTGCG CGCAGCCCTC GACGCCCGCC CTCACGATG	180
CTGCCCGGAG CGACAGCGGC AGCTCGGAGC TGAGCGGGGT GGAGCGGAGC GCAGAGCAGT GCAGAGTGAG GAGCAGCTCG TCGGGGAGCT	270
CCGTGTGCC GTCGCGTCCG ACGCACACC ATG GGT GTA CGC AGC CCC CTG TCC GCC TCT GGG CCT CGC GGG GCC GCT GTC	350
Met Gly Val Arg Ser Pro Leu Ser Ala Ser Gly Pro Arg Gly Ala Ala Val	17
CTG GTG CTA CTG CTG CTG CTG GGG CGC GTC GCG CTG TGT TCC GCC GTG GAG GAG AAG AAA GTT TGT CAA GGG	425
Leu Val Leu Leu Leu Leu <u>Leu Leu</u> Gly <u>Arg</u> Val Ala Leu Cys Ser Ala Val Glu Glu Lys Lys Val Cys Gln Gly	42
ACA AAT AAC AAG TTG ACC CAG CTG GGG CAC GTG GAA GAC CAT TTC ACC AGC CTG CAG AGA ATG TAC AAC AAC TGC	500
Thr Asn Asn Lys Leu Thr Gln Leu Gly His Val Glu Asp His Phe Thr Ser Leu Gln Arg Met Tyr Asn Asn Cys	67
GAA GTG GTA CTG AGT AAC CTG GAG ATT ACC TAC GTG GAG CAT AAT CGC GAT CTC ACC TTC CTT AAG ACC ATA CAG	575
Glu Val Val Leu Ser Asn Leu Glu Ile Thr Tyr Val Glu His Asn Arg Asp Leu Thr Phe Leu Lys Thr Ile Gln	92
GAG GTT GCA GGC TAT GTG CTC ATT GCG CTT AAC ATG GTG GAC GTC ATT CCC TTA GAA AAC CTC CAG ATT ATC CGA	650
Glu Val Ala Gly Tyr Val Leu Ile Ala Leu Asn Met Val Asp Val Ile Pro Leu Glu Asn Leu Gln Ile Ile Arg	117
GGG AAT GTG CTT TAT GAC AAC TCT TTT GCC CTG GCA GTT TTA TCC AAT TAC CAC ATG AAT AAA ACC CAG GGA CTT	725
Gly Asn Val Leu Tyr Asp Asn Ser Phe Ala Leu Ala Val Leu Ser Asn Tyr His Met <u>Asn</u> Lys Thr Gln Gly Leu	142
CGA GAG CTG CCA ATG AAA CGG CTA TCA GAA ATT CTC AAT GGA GGT GTT AAA ATC AGC AAC AAC CCC AAA CTG TGC	800
Arg Glu Leu Pro Met Lys Arg Leu Ser Glu Ile Leu Asn Gly Gly Val Lys Ile Ser Asn Asn Pro Lys Leu Cys	167
AAC ATG GAC ACT GTT CTC TGG AAT GAC ATC ATT GAT ACA AGC AGG AAG CCT CTC ACA GTA CTT GAC TTT GCA AGC	875
Asn Met Asp Thr Val Leu Trp Asn Asp Ile Ile Asp Thr Ser Arg Lys Pro Leu Thr Val Leu Asp Phe Ala Ser	192
AAT CTT TCT TCT TGT CCA AAA TGC CAT CCG AAC TGC ACA GAA GAC CAC TGC TGG GGT GCT GGT GAA CAG AAC TGC	950
<u>Asn</u> Leu Ser Ser Cys Pro Lys Cys His Pro <u>Asn</u> Cys Thr Glu Asp His Cys Trp Gly Ala Gly Glu Gln Asn Cys	217
CAG ACT TTA ACA AAA GTC ATC TGT GCC CAG CAA TGC TCT GCG CGG TGC AGA GGA AAG GTG CCC AGT GAC TGC TGC	1025
Gln Thr Leu Thr Lys Val Ile Cys Ala Gln Gln Cys Ser Gly Arg Cys Arg Gly Lys Val Pro Ser Asp Cys Cys	242
CAC AAT CAG TGT GCT GCA GGG TGC ACA GGA CCT CGG GAG AGT GAC TGC CTG GCA TGC CGC AAG TTT CGG GAT GAT	1100
His Asn Gln Cys Ala Ala Gly Cys Thr Gly Pro Arg Glu Ser Asp Cys Leu Ala Cys Arg Lys Phe Arg Asp Asp	267
GCT ACC TGC AAG GAC ACA TGT CCC CCA CTG GTC CTC TAT AAC CCC ACC ACC TAT CAA ATG GAT GTC AAC CCT GAG	1175
Ala Thr Cys Lys Asp Thr Cys Pro Pro Leu Val Leu Tyr <u>Asn</u> Pro Thr Thr Tyr Gln Met Asp Val Asn Pro Glu	292
GGA AAA TAC AGC TTT GGA GCC ACT TGT GTG AGG GAA TGT CCA CAC AAC TAT GTG GTG ACA GAT CAT GGC TCC TGC	1250
Gly Lys Tyr Ser Phe Gly Ala Thr Cys Val Arg Glu Cys Pro His Asn Tyr Val Val Thr Asp His Gly Ser Cys	317
GTT CGC TCG TGT AAT ACT GAT ACT TAC GAA GTG GAA GAA AAT GGT GTT CGG AAG TGT AAA AAA TGT GAT GGG CTA	1325
Val Arg Ser Cys Asn Thr Asp Thr Tyr Glu Val Glu Glu Asn Gly Val Arg Lys Cys Lys Lys Cys Asp Gly Leu	342
TGT AGC AAA GTG TGC AAT GGC ATT GGA ATA GGT GAA CTT AAA GGG ATC CTA TCC ATA AAT GCC ACA AAC ATC GAC	1400
Cys Ser Lys Val Cys Asn Gly Ile Gly Ile Gly Glu Leu Lys Gly Ile Leu Ser Ile <u>Asn</u> Ala Thr Asn Ile Asp	367
TCC TTC AAA <u>AAC</u> TGT ACG AAG ATC AAT GGG GAT GTC AGC ATT CTT CCT GTT GCA TTT CTA GGG GAT GCC TTC ACA	1475
Ser Phe Lys <u>Asn</u> Cys Thr Lys Ile Asn Gly Asp Val Ser Ile Leu Pro Val Ala Phe Leu Gly Asp Ala Phe Thr	392
AAG ACA CTA CCC CTT GAC CCT AAG AAG CTG GAT GTC TTT AGA ACA GTC AAA GAA ATA TCA GGA TTT TTG TTG ATT	1550
Lys Thr Leu Pro Leu Asp Pro Lys Lys Leu Asp Val Phe Arg Thr Val Lys Glu Ile Ser Gly Phe Leu Leu Ile	417
CAG GCC TGG CCT GAT <u>AAT</u> GCT ACT GAT CTC TAT GCT TTT GAA AAT CTG GAG ATT ATC CGA GGC CGA ACC AAG CAG	1625
Gln Ala Trp Pro Asp <u>Asn</u> Ala Thr Asp Leu Tyr Ala Phe Glu Asn Leu Glu Ile Ile Arg Gly Arg Thr Lys Gln	442
CAC GGC CAG TAT TCC CTT GCT GTT GTT AAC TTG AAA ATA CAG TCG TTG GGG CTG CGC TCC CTC AAG GAA ATA AGT	1700
His Gly Gln Tyr Ser Leu Ala Val Val Asn Leu Lys Ile Gln Ser Leu Gly Leu Arg Ser Leu Lys Glu Ile Ser	467
GAT GGA GAC ATT GCC ATT ATG AAG AAC AAG AAC CTC TGC TAT GCT GAC ACC ATG AAC TGG CGC AGC TTG TTT GCT	1775
Asp Gly Ile Ala Ile Met Lys Asn Lys Asn Leu Cys Tyr Ala Asp Thr Met Asn Trp Arg Ser Leu Phe Ala	492
ACT CAG AGT CAG AAA ACA AAA ATT ATA CAG AAC AGA AAT AAA AAT GAT TGT AGT AAG TCA GTC TGC TTT CCT GCA	1850
Thr Gln Ser Gln Lys Thr Lys Ile Ile Gln Asn Arg Asn Lys <u>Asn</u> Asp Cys Ser Lys Ser Val <u>Cys</u> Phe Pro Ala	517
TTT GCA AAA GCT CAT AAT GAA ATG GAA GAG TAA CTTGATG CAAAATTCAT CAGTGATGCT GCTTTACTGA AGACAGTGAG	1930
Phe Ala Lys Ala His Asn Glu Met Glu Glu End	527
GGAAAAGTGG AGTTAGTCAC ACAGGGGATG TGGGCTTTAT GGTGATCAT CATCTGTTAT GGACACAGAA GTTAGCTCTA GACATCAGGC	2020
TTGTTTGTA AAGTTATGCA CACAGCAGTG GTTCTTGAA CAAAATCTAA TTACTTCTCT TTCCAGAATG TGAATTTGCT ACATATTGGG	2110
AATGATCTC CTCTGGTACT GGTTTGGCT TACTGTATGT CTGGTATGTG TTCACAGTGG ATTTTCATTTT AATTTTGAAA GCCCAGAATT	2200
TGCTCCAAAA ATAAAGGCCA GAAGTCAAGT TTGCGGAATT AGG(AAA...)	2243

FIG. 2. Nucleotide sequence and predicted amino acid sequence of the truncated receptor encoded by the avian *c-erbB* 2.6-kb transcript. Three amino acids with a dotted underline and daggers above nine nucleotides in the signal peptide mark amino acids and nucleotides that are not present in the published sequence of the extracellular domain of the chicken EGF-R homolog (32). Another nucleotide and amino acid difference from the published sequence is illustrated at base position 939. The junctions for the signal peptide and the four domains of the extracellular LBD are indicated below the sequence (32). Seven potential sites of N-linked glycosylation are highlighted in bold. The open arrow indicates the point of sequence divergence from the full-length receptor, and the 18 unique amino acids at the carboxy terminus of the truncated receptor are in italics. The polyadenylation signal is underlined.

pBSL173 and pBSL207.2, containing the cDNA inserts, were excised from  $\lambda$ ZAP and sequenced. Both clones were determined to be partial cDNAs, with pBSL173 containing a 1.71-kb insert and pBSL207.3 containing a 1.12-kb insert. A composite sequence of these two cDNA clones and their

translation product is shown in Fig. 2. The structures of the truncated receptor encoded by the 2.6-kb transcript and the full-length receptor are illustrated in Fig. 1A. The sequence of L173 contains 299 nt from the 5' untranslated region and extends to the middle of domain III of the LBD. The

sequence of clone L207.3 starts in the middle of domain II of the LBD (nt 1173; Fig. 2) and then diverges from the full-length receptor sequence (32) after 10 nt of domain IV (open arrow in Fig. 2) with 416 nt of unique sequence. This unique sequence extends the open reading frame by an additional 18 amino acids (italicized in Fig. 2). The rest of the 416-nt unique sequence corresponds to the 3' untranslated region, and it contains a consensus polyadenylation signal (underlined in Fig. 2) 28 nt upstream of a stretch of 40 A nucleotides. A computer search of the 3' divergent region with sequences in GenBank revealed no significant homology to the other known sequences. Within the homologous region, there are a total of 10 nucleotide differences between our cDNA clones and the published cDNA of the full-length receptor (32). The region of the cDNA encoding the signal peptide has nine additional nucleotides which code for three additional amino acids. The tenth nucleotide change (nt 939) results in the substitution of a glutamic acid residue for a glutamine in domain II. Nucleotide and amino acid differences from the published sequence could possibly represent allelic differences in *c-erbB* (46).

**Exon-intron analysis reveals alternative RNA processing.** The structure of the cDNA clone encoding the 2.6-kb transcript suggested that alternative RNA processing of primary transcripts from the avian *c-erbB* gene or transcription from a related gene was responsible for generating this truncated receptor. We screened genomic DNA clones from the avian *c-erbB* locus to determine the origin of this truncated receptor cDNA. Specifically, a  $^{32}$ P-labeled probe from the 3' divergent end of the 2.6-kb cDNA (probe 3; Fig. 1A) was used to screen a panel of *Eco*RI-digested overlapping genomic  $\lambda$ EMBL clones of the avian *c-erbB* gene (45). Probe 3 hybridized exclusively to a 2.7-kb *Eco*RI fragment that was subcloned into Bluescript (Stratagene) to make pBSg2.7RI. The insert of pBSg2.7RI was sequenced and found to contain three exons (exons 11, 12, and 13) of the full-length receptor (12) and the 3' divergent region of the 2.6-kb transcript. A schematic illustration of the genomic 2.7-kb *Eco*RI fragment and the splicing pattern is shown in Fig. 1B. All three of the splice donor and acceptor sites for the full-length receptor exons fit the consensus sequence. Therefore, generation of the 3' divergent end of the 2.6-kb transcript cannot be explained simply as the result of a poor splice donor-acceptor pair between exons 12 and 13.

**Analysis of *c-erbB* mRNA expression.** We and others (24, 38, 42, 45, 59) have analyzed the transcriptional pattern of the avian *c-erbB* gene with probes derived from the intracellular tyrosine kinase domain. There are three transcripts, 12, 8.6, and 5.8 kb (Fig. 3, lane 1) expressed at low levels in most avian tissues (45). Using a  $^{32}$ P-labeled cDNA fragment (probe 2; Fig. 1A) from the extracellular LBD of the avian receptor as a probe, we see an additional small 2.6-kb transcript (Fig. 3, lane 2). Here we demonstrate that the smallest transcript (lane 3) is exclusively detected with a cDNA probe from the 3' divergent end of the cloned 2.6-kb transcript (probe 3; Fig. 1A). Northern (RNA) transfers proved to be insensitive in studying the expression of *c-erbB* transcripts in tissues and embryos. Hence, the more sensitive RNase protection assay was used.

The RNase T<sub>2</sub> protection assay used a 358-nt *Hinc*II-*Xba*I cDNA fragment from the 2.6-kb transcript that was cloned into Bluescript as a probe. A [ $^{32}$ P]rUTP-labeled antisense RNA transcript (XH436 probe; Fig. 1B) was made from an *Xho*I-linearized template by using bacteriophage T7 RNA polymerase. The XH436 probe was used to analyze *c-erbB* expression in 50  $\mu$ g of total cellular RNA samples isolated

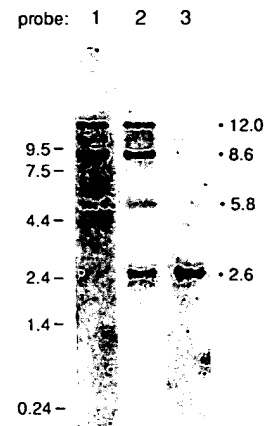


FIG. 3. Northern transfer of poly(A)<sup>+</sup> RNA from CEF cells. Samples of 10  $\mu$ g of poly(A)<sup>+</sup> RNA were electrophoresed through a 1% agarose-formaldehyde gel and transferred to Nytran (Schleicher & Schuell). Probe 1, a 558-nt *Bam*HI fragment from the kinase domain; probe 2, a 954-nt *Bam*HI fragment from the LBD; probe 3, a 283-nt *Xba*I-*Eco*RI fragment from the 3' divergent end of the truncated receptor cDNA (see Fig. 1A). The positions of RNA molecular weight standards (Bethesda Research Laboratories) in kilobases are shown on the left, and the sizes of the four major *c-erbB* transcripts in kilobases are shown on the right.

from adult chicken tissues, CEF cells, and homogenized embryos (Fig. 4). Two RNase-resistant bands are seen in this RNase T<sub>2</sub> protection assay. The 358-nt protected band corresponds to the 2.6-kb transcript, while the 178-nt protected band represents most of exon 12 of the full-length receptor transcripts. The relative level of *c-erbB* expression is lower than the expression level of glyceraldehyde-3-phosphate dehydrogenase (data not shown). Steady-state levels of *c-erbB* expression in adult tissue were the highest in liver, skin, and kidney. Moderate levels were present in testes, adipose tissue, blood vessels, heart, gizzard, and spleen. Low steady-state levels were found in skeletal muscle, bursa, and brain, and very low levels were found in adult bone marrow. Higher levels of *c-erbB* expression were seen in samples of total or fractionated 1-day-old chicken bone marrow than in adult bone marrow (21). In homogenized chicken embryos, *c-erbB* expression was detected in day 4 embryos (earliest time point analyzed) and increased during development (Fig. 4B). The increase in *c-erbB* expression during development is most likely due to the development and growth of high-*c-erbB*-expressing organs such as the liver, kidney, and skin that represent a larger fraction of the embryo by weight during development (48). Densitometric scans of Northern blots and RNase protection assays revealed that the 2.6-kb transcript represented  $25 \pm 5\%$  of the steady-state level of *c-erbB* transcripts. Also, there may be subtle differences in the expression of the truncated receptor transcript versus the full-length receptor transcripts in certain tissues (e.g., adipose tissue and bursa).

**Expression of the truncated receptor in COS1 and CEF cells.** The cDNA encoding the 2.6-kb transcript was cloned into transient and stable expression vectors to confirm the identity of the truncated receptor as the putative translation product of the cDNA. A 1,748-nt *Sst*I-*Xba*I cDNA fragment (2.6SX) that contains 33 nt of the 5' untranslated region and the complete truncated receptor coding region followed by 181 nt of the 3' untranslated region was cloned into expres-

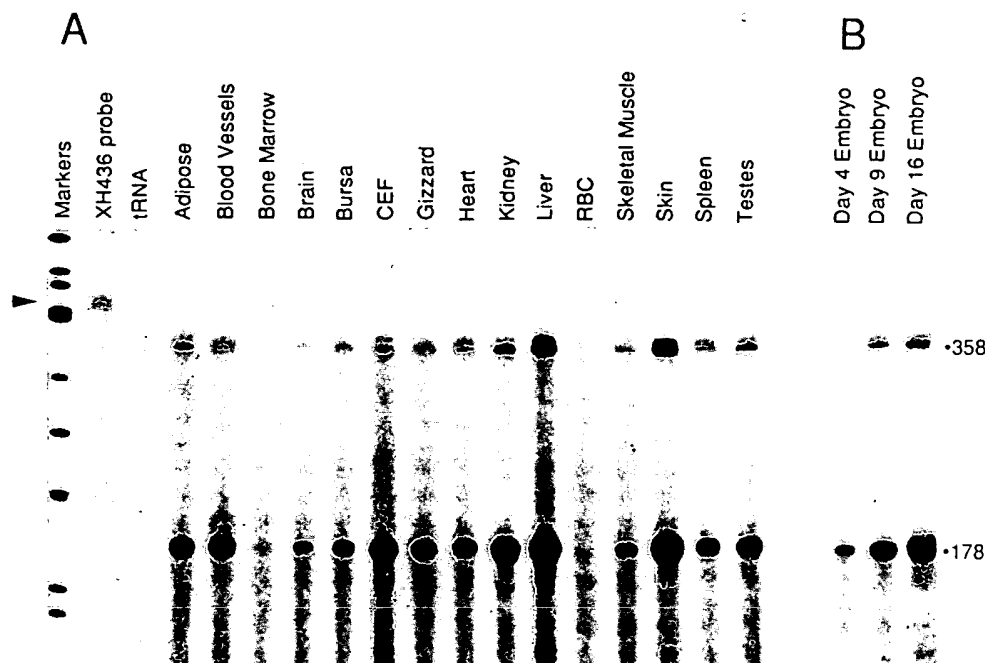


FIG. 4. RNase  $T_2$  protection analysis of *c-erbB* expression. A) [ $^{32}$ P]rUTP-labeled antisense RNA probe, XH436 (see Fig. 1B), was used to analyze *c-erbB* expression in an RNase protection assay. The arrowhead marks the position of the XH436 probe, and the markers are end-labeled  $\phi$ X174 *Hinf*I-digested fragments. The two protected bands are a 358-nt fragment corresponding to the 2.6-kb transcript and a 178-nt fragment corresponding to the full-length receptor transcripts. (A) Expression of *c-erbB* in 50- $\mu$ g total RNA samples from adult chicken tissues and CEF cells. RBC, erythrocytes. (B) Developmental expression of *c-erbB* in 50- $\mu$ g total RNA samples from homogenized chicken embryos.

sion vectors that contain the SV40 late promoter (pSVL; Pharmacia-LKB) and the CMV promoter (pRcCMV; Invitrogen). The results of transiently transfecting COS1 cells with the pSVL2.6SX construct are shown in Fig. 5A. These transfected COS1 cells produce a 70-kDa protein that can be specifically immunoprecipitated with a polyclonal antiserum ( $\alpha$ LBD) from both conditioned medium and cells. Additionally, samples of conditioned medium from pSVL2.6SX-transfected COS1 cells, but not untransfected COS1 cells, are positive in enzyme-linked immunosorbent assays with an antipeptide serum directed against the unique 18 carboxy-terminal residues of the truncated chicken receptor (data not shown). In pSVL2.6SX-transfected COS1 cells incubated with tunicamycin, an inhibitor of N-linked glycosylation, a protein with an apparent size of approximately 52 kDa can be specifically immunoprecipitated with  $\alpha$ LBD. The observed 52-kDa size of this aglycosylated protein is close to a computer-predicted size of 54.9 kDa for the unglycosylated translation product of the 2.6-kb *c-erbB* transcript without a signal peptide.

We have also stably expressed the cDNA of the 2.6-kb transcript in CEF cells to study the possible physiological role of this truncated receptor in a more natural setting. For this purpose, pRcCMV (Invitrogen), an expression vector that contains the CMV promoter and a selectable neomycin resistance gene, was used. Immunoprecipitation of untransfected and pRcCMV2.6SX-transfected CEF cells was performed (Fig. 5B). Stably transfected CEF cells overexpress a 70-kDa truncated receptor in conditioned medium and within cells that is not detectable in untransfected CEF cells under similar conditions. Not only is the native 170-kDa EGF-R homolog detected in CEF cells, but we also see a

~95-kDa  $\alpha$ LBD-cross-reactive doublet of proteins. We have previously identified these ~95-kDa products in CEF cells (38); they are possibly proteolytic products of the full-length chicken EGF-R homolog or perhaps the products of other *erbB*-related transcripts. Interestingly, the immunoprecipitations with  $\alpha$ LBD (Fig. 5) show that most of the 70-kDa truncated receptor is within cells. These data suggest a complex relationship between expression of the alternate *c-erbB* transcript and expression and processing of the protein product. This interesting mechanism of what is apparently posttranscriptional regulation of expression is currently under investigation. However, these results with transfected COS1 and CEF cells demonstrate that the 2.6-kb transcript from the avian *c-erbB* gene encodes a secreted, 70-kDa truncated receptor molecule.

**Functional analysis of the truncated receptor.** Two experiments were undertaken to determine whether the 70-kDa truncated receptor could bind TGF $\alpha$  and have a possible regulatory function. First, we demonstrate that the truncated receptor can be coimmunoprecipitated with TGF $\alpha$  by a nonneutralizing monoclonal antibody against TGF $\alpha$  (Fig. 6). Lanes 2 and 3 show that the 70-kDa truncated receptor is immunoprecipitated from conditioned medium by  $\alpha$ LBD in transfected but not untransfected COS1 cells as in Fig. 5A. In lanes 4 and 5, the samples of conditioned medium were first incubated with 1 ng of recombinant human TGF $\alpha$  (GIBCO) per ml for 30 min at room temperature and then immunoprecipitated with the nonneutralizing monoclonal antibody against human TGF $\alpha$  (Oncogene Science). Again, only in the conditioned medium of pSVL2.6SX-transfected COS1 cells is the 70-kDa truncated receptor able to be coimmunoprecipitated with TGF $\alpha$ . Lane 6 demonstrates the

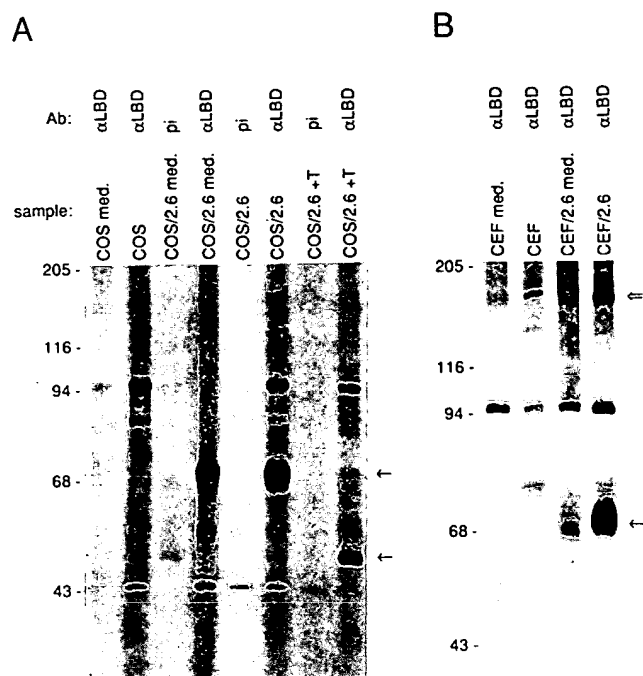


FIG. 5. Immunoprecipitation of a 70-kDa truncated receptor from transfected COS1 and CEF cells. (A) Cells were metabolically labeled with [ $^{35}$ S]cysteine for 16 h and immunoprecipitated with a polyclonal antiserum specific for the extracellular LBD of the avian *c-erbB* gene product ( $\alpha$ LBD) (38) or with a preimmune serum (pi) control. Ab, antibody. The top arrow on the right marks the 70-kDa truncated receptor, and the lower arrow on the right marks the ~52-kDa truncated receptor produced in cells pretreated with tunicamycin (+T), an inhibitor of N-linked glycosylation. COS, COS1 cells; COS med., COS1 conditioned medium; COS/2.6, COS1 cells transiently transfected with pSVL2.6SX; COS/2.6 med., conditioned medium from COS1 cells transiently transfected with pSVL2.6SX. (B) Cells were metabolically labeled with [ $^{35}$ S]cysteine for 22 h and immunoprecipitated with  $\alpha$ LBD. The open arrow on the right marks the position of the native 170-kDa receptor in the CEF and CEF/2.6 lanes. The lower arrow on the right highlights the position of the 70-kDa truncated receptor. CEF, CEF cells; CEF med., CEF conditioned media; CEF/2.6, CEF cells stably transfected with pRcCMV2.6SX; CEF/2.6 med., conditioned medium from CEF cells stably transfected with pRcCMV2.6SX. Positions of molecular weight markers in kilodaltons are indicated on the left of both panels.

identity of the 70-kDa protein as the 70-kDa truncated receptor, since it can be immunodepleted by  $\alpha$ LBD and protein A-Sepharose treatment from a sample of conditioned medium from pSVL2.6SX-transfected COS1 cells before coimmunoprecipitation with TGF $\alpha$ . These results demonstrate that the truncated receptor from transfected COS1 cells is the 70-kDa protein that is coimmunoprecipitated with MAb-TGF $\alpha$ , and therefore this truncated receptor can bind to TGF $\alpha$ . Attempts to quantify the affinity of the 70-kDa truncated receptor from transfected COS1 cells for TGF $\alpha$  have been inconclusive because of the high levels of non-specific binding of  $^{125}$ I-TGF $\alpha$  present in samples of concentrated medium from both transfected and untransfected COS1 cells. This study awaits the purification of the 70-kDa truncated receptor.

This truncated receptor may indeed possess a physiological function as an antagonistic molecule that can block ligand-dependent activation of the native full-length chicken

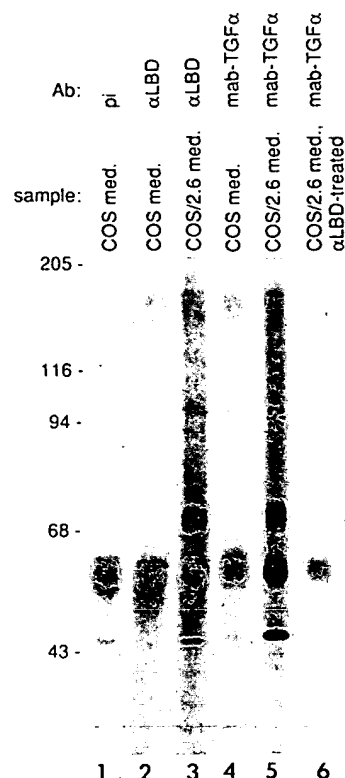


FIG. 6. Coimmunoprecipitation of the 70-kDa truncated receptor with a nonneutralizing monoclonal antibody to human TGF $\alpha$ . Conditioned medium (med.) from cells metabolically labeled with [ $^{35}$ S]cysteine for 16 h was immunoprecipitated with  $\alpha$ LBD or preimmune (pi) serum control or MAb-TGF $\alpha$  (Ab-1; Oncogene Science). Conditioned medium from untransfected COS1 cells (COS) or pSVL2.6SX-transfected COS1 cells (COS/2.6) either was immunoprecipitated with preimmune serum or  $\alpha$ LBD as for Fig. 5 (except using protein G-Sepharose) or was first incubated with 1 ng of recombinant human TGF $\alpha$  (GIBCO) per ml for 30 min at room temperature and then immunoprecipitated with MAb-TGF $\alpha$  and protein G-Sepharose. Lane 6 contained conditioned medium that was first immunodepleted of the 70-kDa truncated receptor by treatment with  $\alpha$ LBD and protein A-Sepharose for 1 h. Then the depleted medium was incubated with 1 ng of TGF $\alpha$  per ml and immunoprecipitated with MAb-TGF $\alpha$  and protein G-Sepharose. The arrow on the right marks the position of the 70-kDa truncated receptor, and positions of molecular weight markers in kilodaltons are indicated on the left. Ab, antibody.

EGF-R homolog in soft agar colony assays (Fig. 7). CEF cells express moderate levels of the full-length receptor encoded by the *c-erbB* gene and can grow in soft agar upon addition of TGF $\alpha$  (2). To test the ability of the truncated receptor to inhibit ligand-dependent soft agar colony formation, CEF cells were transfected with the 2.6-kb *c-erbB* expression construct, pRcCMV2.6SX, or with vector alone, and stable transfectants were selected with G418 (GIBCO). The pooled population of stably transfected CEF cells was then suspended in complete medium containing agar (see Materials and Methods) with or without exogenously added recombinant human TGF $\alpha$  (GIBCO). The vector-alone-transfected CEF cells form soft agar colonies in a dose-dependent manner (Fig. 7, CEF/pRcCMV). Over 95% of CEF cells transfected with the 70-kDa truncated receptor were blocked in their ability to form colonies in soft agar (Fig. 7, CEF/pRcCMV2.6SX). Also, the pRcCMV2.6SX-



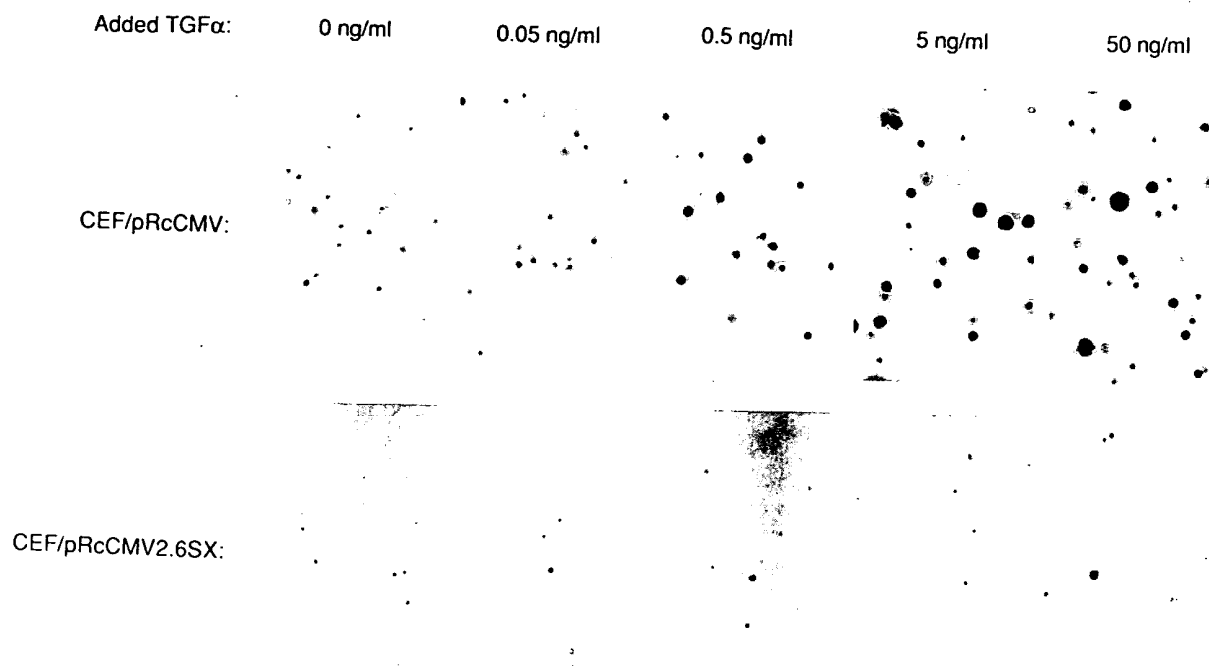


FIG. 7. TGF $\alpha$ -dependent soft agar colony assay of stably transfected CEF cells. CEF cells were transfected with vector alone (pRcCMV) or with the vector that expressed the 2.6-kb transcript (pRcCMV2.6SX) and selected with G418 (GIBCO) for 10 days. The cells were allowed to recover for 4 days, and then  $10^4$  cells per well were cultured in soft agar with the corresponding amount of recombinant human TGF $\alpha$  (GIBCO) for 2 weeks as described in Materials and Methods.

transfected CEF cells had no small background colonies in complete medium alone (Fig. 7, TGF $\alpha$  at 0 ng/ml) and remained as single cells. Exogenously added 70-kDa truncated receptor (in the form of concentrated conditioned medium from transfected COS1 cells) could not be tested in this assay because concentrated conditioned medium from both transfected and untransfected COS1 cells caused the formation of ligand-independent soft agar colonies by CEF cells. Together, the results of these two experiments strongly suggest that the 70-kDa truncated receptor can bind TGF $\alpha$  and can act as an antagonistic molecule toward ligand activation of the full-length 170-kDa receptor.

## DISCUSSION

In this study, we report the cloning of the cDNA of the 2.6-kb transcript from the avian *c-erbB* gene and provide evidence that this transcript encodes a secreted, truncated receptor molecule that can bind TGF $\alpha$  and block ligand-dependent soft agar colony formation in CEF cells. We further demonstrate that alternative processing of primary *c-erbB* transcripts leads to the formation of the small 2.6-kb transcript. Specifically, a consensus polyadenylation signal between exons 12 and 13 of the full-length receptor is utilized to generate the truncated receptor transcript (Fig. 1B). The only other reported truncated receptors that appear to use this method (use of an alternative polyadenylation signal within an intron to replace the 3' end) are the rat GH receptor (8) and the human FGF receptor (28). The other alternatively spliced truncated receptors appear to either splice in a new exon that contains a translational stop codon and polyadenylation signal as in the rat EGF-R (44) or splice out an apparent exon that contains the transmembrane

domain of the full-length receptor and shifts the reading frame (3, 22, 23, 41, 47, 54).

Translation of the 2.6-kb *c-erbB* transcript would generate a secreted, truncated receptor that contains all of the extracellular domains I through III and three residues of domain IV followed by 18 unique amino acids before termination at a translational stop codon (Fig. 1A and 2). The truncated chicken receptor is structurally distinct from the truncated EGF-Rs in normal rats and the human epidermoid carcinoma A431 cell line (Fig. 8). The major difference between the chicken receptor and the other two truncated receptors is that the chicken truncated receptor lacks most of the cysteine-rich domain IV (Fig. 8). Although formation of the

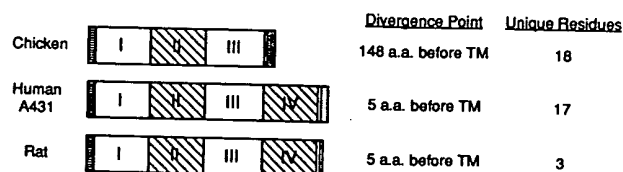


FIG. 8. Schematic diagram of secreted, truncated EGF and TGF $\alpha$  receptors. A structural comparison of the truncated receptors encoded by the 2.6-kb transcript from the avian *c-erbB* gene, the 2.8-kb transcript from the amplified and rearranged EGF-R locus in human A431 cells (57), and the 2.7-kb transcript from the rat EGF-R locus (44) is illustrated. The divergence point in amino acids (a.a.) relative to the start of the transmembrane (TM) domain and the number of unique amino acid residues on the carboxy-terminal end of the truncated receptors are shown. Horizontally hatched boxes, signal peptides; diagonally hatched boxes, cysteine-rich domains; stippled boxes, unique carboxy-terminal residues; I to IV, subdomains within the extracellular LBD.

truncated receptors in chickens and rats (44) is due to normal alternative RNA processing events and not the apparent result of a DNA recombinational event as in human A431 cells (40), the truncated EGF-Rs produced in rats and human A431 cells are structurally similar. Both of these ~95-kDa (the reported size of the human truncated receptor varies from 95 to 117 kDa) truncated receptors contain all four domains of the extracellular LBD, and they diverge from their full-length receptors at a point located a few amino acids upstream of the transmembrane domain. The truncated human EGF-R does bind EGF, although there are disagreements over the affinity (6, 25, 60), and it can be cocrystallized with EGF (25). We provide two pieces of evidence that suggest that the 70-kDa truncated chicken receptor can bind TGF $\alpha$ . First, the 70-kDa truncated receptor can be coimmunoprecipitated with TGF $\alpha$  by a monoclonal antibody against TGF $\alpha$  (Fig. 6). Furthermore, the 70-kDa protein can be specifically immunodepleted with the polyclonal  $\alpha$ LBD serum from the transfected COS1 conditioned medium, which strongly suggests that the coimmunoprecipitated 70-kDa protein is the truncated chicken receptor. Second, transfected CEF cells that overexpress the 70-kDa truncated receptor are blocked in their ability to form TGF $\alpha$ -dependent colonies in soft agar assays (Fig. 7). Although the precise mechanism by which the truncated receptor blocks soft agar colony formation in transfected CEF cells is unknown, the truncated receptor could be acting as a competitor for ligand or as a molecule that blocks dimerization of full-length receptors (reviewed in reference 58) or even as an intracellular antagonistic molecule. These apparent binding results suggest that domain IV of the extracellular LBD which is absent from the 70-kDa truncated receptor is not required for ligand binding. This assertion agrees with results of affinity labeling studies (31, 62) and chicken-human receptor chimeras (30) that demonstrate that the major ligand-binding site resides within domain III. Furthermore, the 200-fold-higher affinity of the chicken receptor for TGF $\alpha$  over EGF resides in 35 amino acid differences between the chicken and human receptors in domain III (30, 32). Several studies (30, 32, 43) have demonstrated the preference of the chicken receptor encoded by the avian *c-erbB* gene for TGF $\alpha$  and not EGF. Perhaps the avian *c-erbB* gene encodes the chicken TGF $\alpha$  receptor and not the EGF-R. Low-stringency Southern blots of restricted chicken genomic DNA probed with cDNA fragments from the coding domain of human EGF and TGF $\alpha$  precursors (10, 37) revealed that only the TGF $\alpha$  probe weakly hybridizes to chicken genomic DNA fragments (data not shown). The answer to this question will be resolved only when the native chicken ligand is definitively identified and cloned.

Truncated, soluble receptors have been reported for a wide variety of growth factor receptor systems. Proteolytic cleavage of native full-length receptors and transcription of alternatively spliced and processed RNA transcripts appear to be the two mechanisms used to generate these truncated, soluble receptor forms. The wide distribution of these truncated, soluble receptors suggests that these molecules may have common physiological roles. For example, studies of the GH-binding protein suggest that this truncated receptor molecule may act as a reservoir for GH in the serum that increases the half-life of GH and alters the distribution of GH in the body (7). Other reports of possible functions suggest that truncated receptors can act as antagonistic molecules that inhibit full-length receptor function. Basu and colleagues (6) have reported that the truncated human EGF-R from A431 cells can inhibit ligand activation of the full-length

receptor although the truncated receptor does not appear to be acting simply as a competitive inhibitor. Two recent reports of genetically engineered soluble truncated receptor forms also support the hypothesis of truncated receptors acting as antagonists of full-length receptor function. A recombinant soluble form of the platelet-derived growth factor (PDGF)  $\beta$  receptor can specifically bind to and dimerize with the BB-homodimeric form of PDGF BB-PDGF, thereby inhibiting binding to BB-PDGF and activation of the PDGF  $\beta$  receptor (20). Recombinant soluble forms of the murine IL-1 and IL-4 receptors can specifically inhibit IL-1- and IL-4-induced proliferation and differentiation of B cells in vitro (39). Also, a recombinant membrane-bound truncated form of the FGF receptor can act as a dominant negative mutant in *Xenopus* embryos and disrupts the formation of mesoderm (1). Further characterization of the 70-kDa truncated receptor from the avian *c-erbB* gene will be required to determine the function(s) of this specific truncated receptor molecule.

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